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# Extraction, Separation and Spectrophotometric Determination of Hg(II) using 2- [(3-hydroxy-4-nitrophenyl) methylidene] hydrazine-1-carbothioamide (HNMHC) as a chromogenic reagent.

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## Abstract

A simple, selective, and sensitive extractive spectrophotometric technique was successfully developed for the quantitative determination of mercury(II) using 2- [(3-hydroxy-4-nitrophenyl) methylidene] hydrazine-1-carbothioamide (HNMHC) as a chromogenic reagent. Mercury (II) formed a stable complex with HNMHC in the presence of potassium iodide, which was quantitatively extracted in to n-butanol and measured at 368 nm. Experimental parameters affecting complex formation and extraction, including potassium iodide concentration, reagent concentration and extraction solvent were enhanced. Under the optimized condition, beer's law obeyed over the concentration range of 5-75 μg $mL^{-1}$ with molar absorptivity of $2.57 \times 10^4$ L $mol^{-1}$ $cm^{-1}$ and Sandell's sensitivity of 0.0156 μg $cm^{-2}$. The detection limit of the method as 0.0654 μg $mL^{-1}$. the proposed method shows good selectivity in the presence of several foreign ions, while interference from selected ions was effectively masked. The developed method was successfully applied for the separation and quantification of Hg(II) in binary and ternary synthetic mixtures containing different associated metal ions. The applicability of the method was further demonstrated by analyzing environmental samples, including river water, lake water and groundwater. The developed method is rapid, economical, accurate and suitable for trace level determination of mercury (II)

## Keywords

Mercury (II), HNMHC, spectrophotometric method, environmental samples

## Introduction

Forensic Elemental mercury is existing in the Earth's crust at a concentration of approximately 0.5 parts per million [1]. In natural ecosystems, mercury is the most hazardous element. Mercury, also called as quicksilver, is a distinctive chemical element characterized by several exceptional physical properties. It is the only metal that remains in a liquid phase at room temperature condition. It has an atomic number of 80 and an atomic mass of 200.59 g/mol. It possesses a remarkably high density, approximately 13.5 times that of water. Additionally, it exhibits a low melting point of −38.8 °C and a boiling point of 356.7 °C, underscoring its unusual thermal behavior among metallic elements.[2]

This occurs through several industries including the pulp and paper industry, gold and silver mining, electrical industry, paints industry, fungicides pharmaceuticals, chlor-alkali industries, plastic industries and petroleum refining industries [3-6] it's also used in antiseptics, reagents catalyst and batteries in healthcare [7]. Mercury is highly volatile, which means it can easily enter the human environment and pose serious health risks. Exposure to mercury can damage the nervous system, leading to symptoms such as irritability, paralysis, insanity, blindness, and even genetic damage that may cause developmental defects. A common example of serious mercury toxicity is Minamata disease, which results in severe neurological problems. People affected may experience mental disturbances, instability in equilibrium, difficulty with speech, vision, and sense hearing, as well as trouble swallowing. In severe cases, it can lead to brain degeneration and even death.[8]

Mercury is also considered one of the most dangerous metal contaminants that can be found in the foods we eat every day.[9] Mercury becomes toxic because it builds up in body tissues over time through

contaminated food. Once inside the body, it can cause serious harm to the central nervous system, as well as damage the brain, kidneys, and lungs. It can also interfere with the healthy development of a fetus.[10]. The level of mercury toxicity is influenced by its chemical state. Inorganic mercury tends to bind strongly to certain proteins in the body and usually builds up in the kidneys. In contrast, organic mercury is more likely to affect the brain, where it can accumulate and cause damage.[11] Mercury and its compounds produced serious diseases including leukemia. Much of the mercury found in the environment comes from human activities, and it can be detected in different environmental samples. In natural water sources, mercury is usually present in very small amounts, but lakes and rivers near industrial areas often serve as key indicators of mercury pollution. Because of these risks, there is a strong need to develop new methods for detecting mercury that are selective, effective, and affordable. [12]

A variety of analytical techniques, including cold-vapor atomic absorption spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma-atomic emission spectrometry (ICP-AES), [13, 14] Chemiluminescence, Electrochemical Analysis, and all other methods[15] have been employed to selectively determine Hg (II). Currently, some simple methods are active as inexpensive and fast techniques for determination of Hg (II) such as colorimetry, fluorimetry and voltametric methods [16]. Nevertheless, these techniques have several drawbacks, such as the requirement for exclusive equipment, high operating costs, and skilled professionals to carry out the analysis.

Some authors have reported on the Mercury (II) was determined by extractive spectrophotometry using variety of reagents [9-34]. However, highly selective reagents have been reported rarely. Table 1 displays a comparative analysis of recently developed methodologies and established techniques applied for the extractive identification of mercury (II). This study purposes to develop a highly efficient spectrophotometric method for the determination of mercury (II) at micro-level concentrations, utilizing 2- [(3-hydroxy-4-nitrophenyl) methylidene] hydrazine-1-carbothioamide (HNMHC) as the analytical reagent. The methodology is designed to be simple, sensitive, and quick for the extraction, separation and spectrophotometric determination of mercury (II).

**Table 1: Comparative study of the superiority of the present method over reported**

| **Name of the Reagent** | **λmax** | **pH** | **Beer's law range mg/ml** | **Molar Absorptivity (L mol$^{-1}$ cm$^{-1}$)** | **Extraction / Aqueous** | **Ref.** |
|---|---|---|---|---|---|---|
| N'',N'''-bis[(E)-(4-fluorophenyl)methylidene] Thiocarbonohydrazide bis-(4-fluoroPM)TCH. | 375 | 1.7-3.7. | 0.25 to 3.5 | $0.50127\times10^5$ | Iso amyl acetate | [9] |
| 4,5-bis(4-methoxyphenyl) imidazole | 546 | -- | 0.5-10 | $7.963\times10^3$ | Aqueous | [10] |
| 2-Acetylpyridine Thiosemicarbazone | 351 | 6.0 | 0.240-2.407 | $5.4\times10^4$ | Aqueous | [11] |
| 5 methylthiophene–2-carboxaldehyde ethylenediamine (MTCED) | 385 | 2.0 | 0.83-8.6 | $5.58\times10^4$ | Aqueous | [12] |
| Thiokrown ether and Bromocresol Green | 420 | 3.5 | 0.5-12.0 | 0.14 | Chloroform | [16] |
| Tetraphenyl Phridinium Perchlorate | 310 | 0.5M $H_2SO_4$ | 0.04-0.5 | $2.64\times10^4$ | Isopentyl acetate | [17] |
| 1,5–Diphenylthiocarbazone (Dithizone) | 490 | 0.07-0.17 M $H_2SO_4$ | 0.05-10 | $5.02\times10^4$ | Aqueous | [18] |
| 3-methylthiophne-2-carbaxaldehyde thiosemicarbazone (3- | 365 | 8.0 | 0.8-8.02 | $1.87\times10^4$ | Aqueous | [19] |

| | | | | | | |
|---|---|---|---|---|---|---|
| MTAT) | | | | | | |
| Diacetyl Monoxime Isonicotinoyl hydrazone (DMIH) | 351 | 5.5 | 1.0-12.0 | $2.23 \times 10^4$ | Aqueous | [20] |
| Diphenyl thiocarbazone (dithizone) | 488 | 0.8M $H_2SO_4$ | 0.1-25 | $2.5 \times 10^4$ | Aqueous | [21] |
| 6-Hydroxy-3-(2-oxoindolin-3-ylideneamino) | 505 | 4-6 | 0.2-2.0 | $4.0 \times 10^4$ | Dioxane | [22] |
| 3,4-Dihydroxybenzaldehydetiosemic-arbazone (DHBTSC) | 375 | -- | 5.01-50.14 | $3.25 \times 10^4$ | Aqueous | [23] |
| Benzyl 2-pyridylketone 2-quinolylhydrazone (BPKQH) | 475 | 9.0-10.4 | | $5.01 \times 10^4$ | Aqueous | [24] |
| 2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol | 565 | 8.0-10.0 | 0.02-1.00 | $1.10 \times 10^5$ | Aqueous | [25] |
| Variamine Blue B | 605 | 2.5-4 | $3.2x10^{-6}$ to $2.2x10^{-5}$ | $4.03 \times 10^4$ | Nitrobenzene | [26] |
| Potassium benzyl xanthate (KBX) | 375 | 8.0to 12.0 | 1.0 to 20 | $2.609 \times 10^{-3}$ | Methyl isobutyl ketone | [27] |
| N-p-chlorophenylbenzohydroxamic acid | 388 | 9.0 | 20 to 400 | $6.0 \times 10^2$ | Chloroform | [28] |
| 1,2,4,6_tetraphenylpyridinium perchlorate (TPPP) | 310 | 5M $H_2SO_4$ | 0.04-0.5 | $2.63 \times 10^4$ | isopentyl acetate. | [29] |
| N-phenylbenzohydroxamic acid (PBHA) | 550 | 6.8 | 0-10 | $6.0 \times 10^3$ | Chloroform | [30] |
| lodonitrotetrazolium chloride (INT) | 260 | KI | 0.1-1.2 | $(1.00 \pm 0.09) \times 10^5$ | dichloroethane | [31] |
| Rhodamine B | 556 | -- | 5 to 27 µg Hg(II) / 20 mL | $17.68 \times 10^4$ | benzene | [32] |
| Phenanthroline and Eosin | 552 | 4.5 | 5-30 | $8.0 \times 10^4$ | Aqueous | [33] |
| N'-(1-(pyridin-2-yl) ethylidene) isonicotinohydrazide | 357 | 5.5 | 0.2-2.0 | $5.48 \times 10^4$ | Aqueous | [34] |
| 2- [(3-hydroxy-4-nitrophenyl) methylidene] hydrazine-1-Carbothioamide (HNMHC) | 368 | KI | 5-75 | $2.57 \times 10^4$ | n-Butanol | **Present Method** |

## Experimental:

### Apparatus:

A Double Beam UV–Visible Spectrophotometer 2202, equipped with matched quartz cells of 1 cm path length, was utilized for recording absorbance values. Mass measurements were conducted using a Contech electronic balance (Model CA-123). Prior to use, all calibrated glassware was carefully treated with dilute nitric acid, cleaned with soap solution, and rinsed thoroughly twice with purified water.

### Standard mercury (II) solution:

A standard stock solution of Hg(II) having a concentration of 100 µg $mL^{-1}$ was prepared by dissolving 0.02925 g of mercurous chloride ($Hg_2Cl_2$) in distilled water containing a few drops of concentrated hydrochloric acid. The resulting solution was then diluted to the mark in a 250 mL volumetric flask. The preparation procedure was carried out according to the reported method.[34]

### Synthesis of 2- [(3-hydroxy-4-nitrophenyl) methylidene] hydrazine-1-carbothioamide (HNMHC)

2- [(3-hydroxy-4-nitrophenyl) methylidene] hydrazine-1-carbothioamide was prepared using a modified procedure of the literature [35] [36]. To a mixture of thiosemicarbazide (0.18 g, 0.002 mmol) in 40 mL of ethanol and stirred and glacial acetic acid (2 ml), a solution of 3-hydroxy-4-nitrobenzaldehyde (0.33 g, 0.002 mmol) in ethanol (10 ml) was added dropwise. A reaction mixture was refluxed for 12 hrs. After completion of reaction (as indication by TLC), reaction mixture was cooled to room temperature to afforded yellow crystalline solid. (M.P- $310^0$ C)

The 0.24 gm of 2- [(3-hydroxy-4-nitrophenyl) methylidene] hydrazine-1-carbothioamide was dissolved in 100 mL of dimethylformide and put in a 100 mL calibrated volumetric flask to prepare the solution 0.01 mol $L^{-1}$.

### Recommended procedure

An aliquot containing 25 µg Hg (II), 0.10 mol $L^{-1}$ KI, and 0.004 mol $L^{-1}$ 2- [(3-hydroxy-4-nitrophenyl) methylidene] hydrazine-1-carbothioamide in DMF was transferred into a 10 mL volumetric flask. The resulting mixture was equilibrated with 10 mL of n-butanol. After phase separation, the n-butanol layer containing the Hg(II)–HNMHC complex was collected into a dry beaker. Any residual water was removed by treating with 1.0 g of An. sodium sulphate. The organic phase was then transferred to a 10 mL volumetric flask and diluted up to the mark with n-butanol. The absorbance of the Hg (II)– HNMHC complex was measured at λmax 368 nm compared to a reagent blank.

## Result and Discussion

### Absorption Spectra

The Hg(II)– HNMHC complex demonstrates absorbance between 250 nm and 450 nm, with a peak absorbance (λmax) recorded at 368 nm. At this wavelength, the reagent blank shows no detectable absorption (Figure 1). A

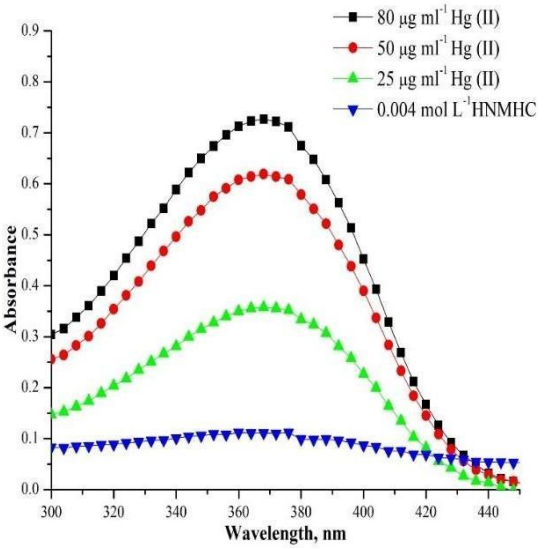


**Figure 1: Absorption spectra of Hg(II)– HNMHC complex**

comprehensive summary of the physicochemical appearance of the Hg(II)– HNMHC complex is presented in Table 2.

**Table 2: Spectral and physico-chemical characteristics of Hg(II)– HNMHC complex**

| ***Characteristic*** | ***Parameters*** |
|---|---|
| *Potassium iodate conc.* | 0.10 mol $L^{-1}$ |
| *Reagent concentration* | 0.004 mol $L^{-1}$ |
| *Equilibration time* | 1 min |
| *Extraction solvent* | n-butanol |
| *λ max* | 368nm |
| *Molar absorptivity* | 2.57 x $10^4$ L mol-1 cm-1 |
| *Sandell's sensitivity* | 0.0156 µg cm-2 |
| *Beer's law range* | 5-75 µg ml-1 |
| *Ringbom's optimum range* | 25-75 µg ml-1 |
| *Limit of detection* | 0.0654 µg ml-1 |
| *Relative standard deviation* | 0.55% |
| *Stoichiometry of the complex* | 1:1 Hg (II): HNMHC |
| *Stability of complex* | >72 hr |
| *Correlation coefficient* | 0.97 |

## Effect of potassium iodide concentration

Potassium iodide (KI) concentration has an important influence on the development of the Hg (II)–HNMHC complex. During the investigation, the KI concentration was systematically varied from 0.02 to 0.20 mol $L^{-1}$. An increase in KI concentration led to corresponding increase in absorbance; however, beyond 0.10 mol $L^{-1}$, no significant change was observed. Consequently, a KI concentration of 0.10 mol $L^{-1}$ was utilized for further investigation. (Figure 2)

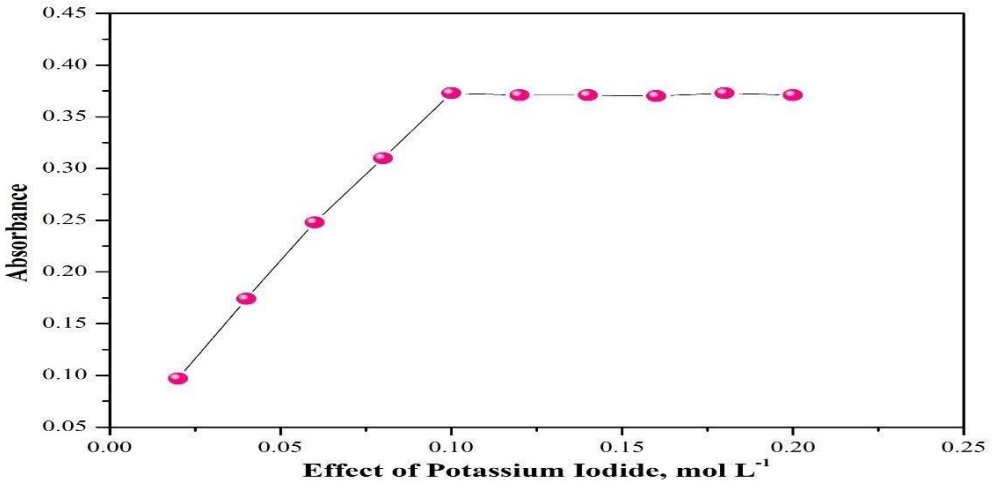


**Figure 2: potassium iodide concentration Variation**

## Effect of reagent concentration

The influence of reagent concentration on the extraction efficiency of 25 µg of mercury (II) was observed by varying the concentration of 2- [(3-hydroxy-4-nitrophenyl) methylidene] hydrazine-1-carbothioamide in DMF within the range of 0.001 to 0.008 mol $L^{-1}$. Optimal complex

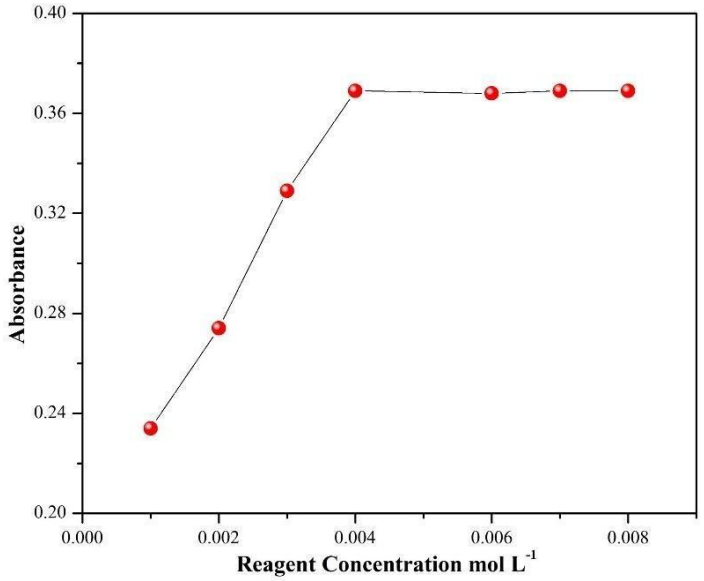


**Figure 3: Reagent Concentration Variation**

formation was achieved using 0.004 mol $L^{-1}$ reagent solution. Furthermore, the use of excess reagent did not adversely impact the extraction process, as demonstrated in Figure 3.

## Effect of extraction Solvent

To assess the suitability of various solvents for the quantitative extraction of complexes such as chloroform, n-butanol, toluene, benzene and ethyl acetate, were evaluated as potential extraction solvents. Among these, n-butanol was identified as the most effective, exhibiting the maximum absorbance of the complex at 368 nm (Figure 4).

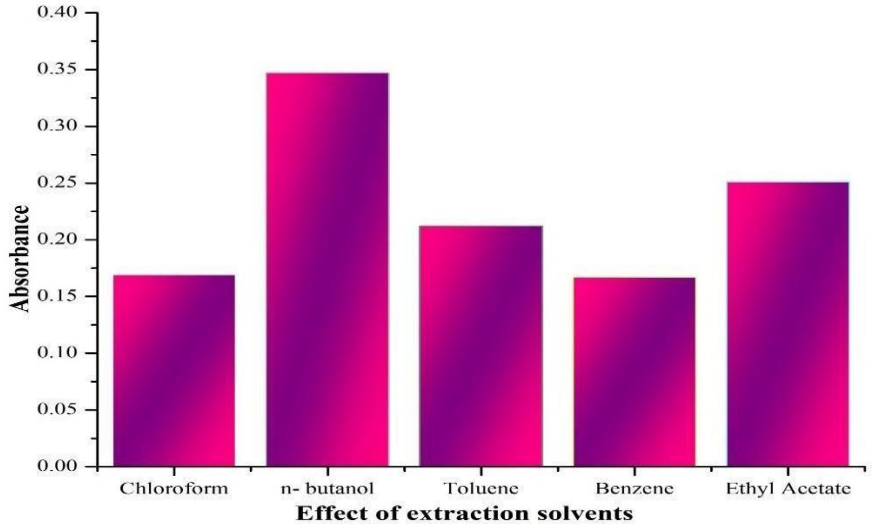


**Figure 4: Effect of extraction solvents on Hg (II)– HNMHC**

## Analytical Characteristics

Beer's law was followed within the concentration range of 5–75 µg $mL^{-1}$ (Figure 5). The method shows molar absorptivity and Sandell's sensitivity as $2.57 \times 10^4$ L $mol^{-1}$ $cm^{-1}$ and 0.0156 µg $cm^{-2}$, respectively. The Calibration curve exhibited good linearity, with correlation coefficient ($R^2$) of 0.9779. Linear regression analysis yielded the equation: y=0.00623x+0.26918.

The Ringbom's plot demonstrated that the most reliable working concentration range for quantitatively analysis was identified as 25–75 µg $mL^{-1}$ (Figure 6).

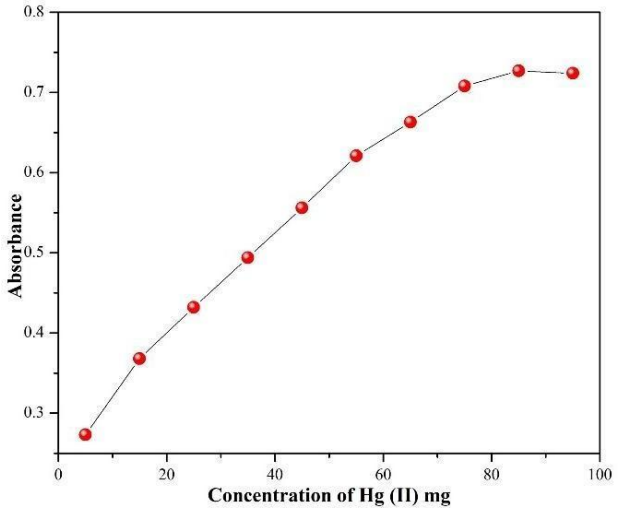


**Figure 5: Beer's law range for Hg (II)– HNMHC complex**

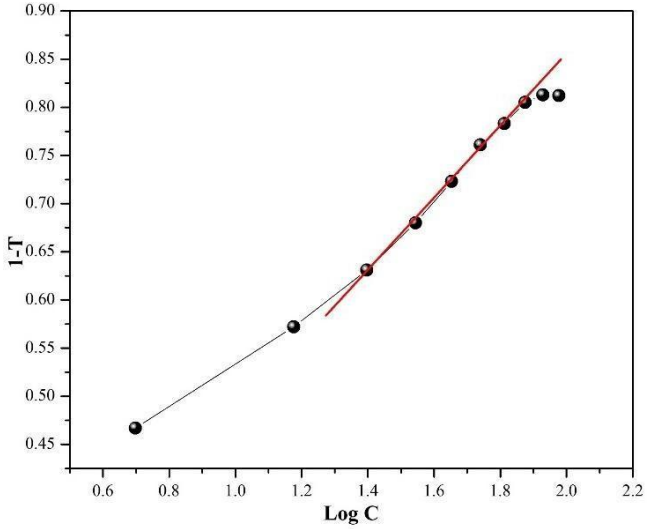


**Figure-6: Ringbom's Plot of Hg (II)– HNMHC complex**

### Stoichiometry of the Hg(II)– HNMHC Complex

The stoichiometry of the Hg(II)– HNMHC complex was examined through Job's method of continuous variation and the mole ratio approach. Consistent outcomes from both techniques indicated that the complex is formed in a 1:1 metal-to-ligand ratio, as illustrated in Figures 7 and 8.

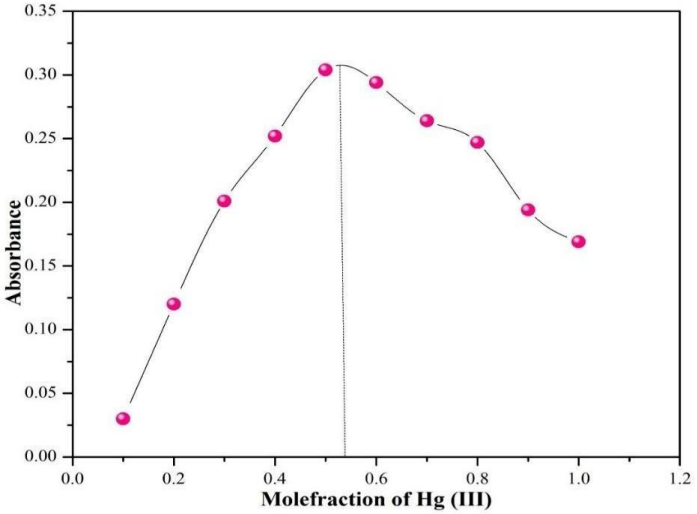


**Figure 7: Job's continuous variation method for Hg (II)– HNMHC complex**

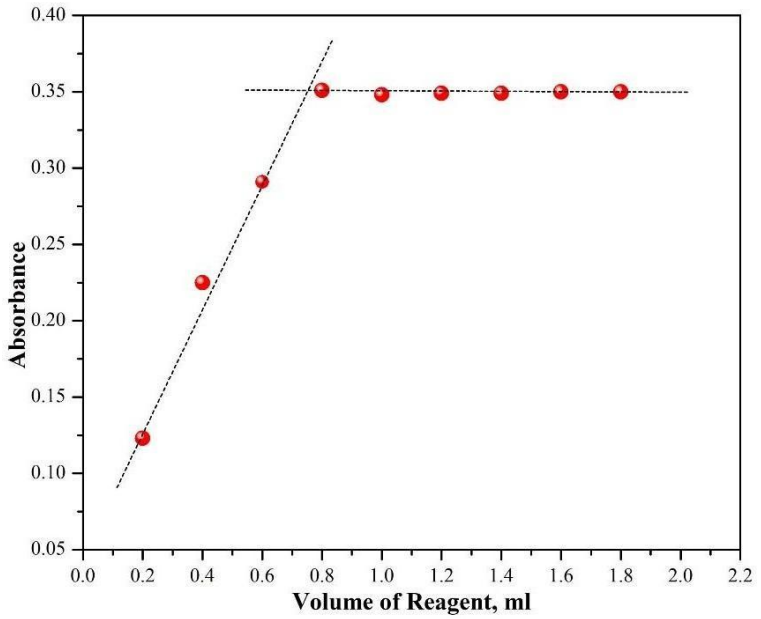


**Figure 8: Mole ratio method for Hg (II)– HNMHC complex**

## Interference Study

By analyzing the effect of different foreign ions, the method's selectivity was assessed. The concentration of ions generating a change in the absorbance of the Hg(II)– HNMHC complex of no more than ±2% was identified as the tolerance limit. by masking with citrate and thiocyanate solutions, interference from Al(III), Cd(II), Cr(III), Zn(II), and Pb(II) ions was effectively removed (Table 3).

**Table 3:** Effect of Foreign Ions

| Foreign ions | Added as | Tolerance Limit (mg) |
|---|---|---|
| Ba (II) | $BaCl_2.2H_2O$ | 0.3 |
| Al (III)*a | $AlCl_3$ | 0.15 |
| Ca (II) | $CaCl_2.2H_2O$ | 0.05 |
| Cd (II)*a | $CdCl_2.H_2O$ | 0.05 |
| Ce (IV) | $(NH_4)_2[Ce(NO_3)_6]$ | 0.05 |
| Co (II) | $COCl_2.6H_2O$ | 0.1 |
| Cr (III)*b | $Cr(NO_3)_3.9H_2O$ | 0.1 |
| Cu (II) | $CuSO_4.5H_2O$ | 0.15 |
| Mg (II) | $Mg(OH)_2$ | 0.1 |
| Mn (II) | $MnSO_4.H_2O$ | 0.15 |
| Mo (VI) | $(NH_4)_6Mo_7O_{24}$ | 0.35 |
| Ni (II) | $NiCl_2.6H_2O$ | 0.2 |
| Sn (II) | $SnCl_2.2H_2O$ | 0.1 |
| Sr (III) | $Sr(No_3)_2$ | 0.35 |
| Zn (II)*b | $ZnCo_3$ | 0.2 |
| Pb(II)*b | $Pb(NO_3)_2$ | 0.05 |
| Bi (III) | $Bi(NO_3)_3.5H_2O$ | 0.1 |
| Bromide | KBr | 0.15 |
| Chloride | NaCl | 0.075 |
| Fluoride | NaF | 0.1 |
| Iodide | KI | 0.1 |
| Nitrate | $KNo_3$ | 0.12 |
| Sulphate | $K2SO_4$ | 0.1 |
| Thiocyanate | KSCN | 0.1 |
| Oxalate | $Na_2C_2O_4^{-2}$ | 0.05 |
| Acetate | $CH_3COONa$ | 0.1 |

*a – Citrate solution (0.05 mg)

*b – SCN- solution (0.1 mg)

## Applications

### Extraction, separation and determination of mercury (II) from binary synthetic mixtures

The suggested approach for the extraction, separation and measurement of mercury (II) in the presence of other metal ions, specifically Ni (II), Co (II), and Pb (II), was applied to binary synthetic mixtures. It was four repetitions
(n). Mercury (II) was quantitatively isolated from the related ions of metals, which continued in the aqueous phase, by using the suggested method. The extracted Hg(II)– HNMHC complex in n-butanol was then measured at 368 nm using spectrophotometry.

Following the quantitative separation of mercury (II), the aqueous phase containing the associated metal ions was evaporated to wet dryness. The solution was then once again evaporated to wet dryness after 2.0 mL of strong hydrochloric acid was added. After cooling and dissolving the residue containing the metal ions in water, the metal ions were measured spectrophotometrically using established techniques.[37] (Table 4)

**Table 4: Extraction, separation and determination of Hg (II) from binary synthetic mixtures.**

Mercury (II): 25 µg $ml^{-1}$, KI: 0.10 mol $L^{-1}$, HNMHC: 0.004 mol $L^{-1}$, λmax: 368 nm

| Metal Ion | Amount Taken (µg) | Recovery[a] (%) | RSD (%) | Complexing Ligand | Ref. |
|---|---|---|---|---|---|
| Hg (II) | 25 | 98.88 | 0.35 | -- | -- |
| Ni (II) | 100 | 99.69 | 0.52 | DMG | 37 |
| Hg (II) | 25 | 98.88 | 0.62 | -- | -- |
| Co (II) | 50 | 99.88 | 0.40 | Nitroso- R- Salt | 37 |
| Hg (II) | 25 | 98.88 | 0.53 | -- | -- |
| Pb (II) | 25 | 99.19 | 0.88 | Dithizone | 37 |

a: Average four determinations

### Extraction, separation and determination of mercury (II) from ternary synthetic mixtures

A 10 mL volumetric flask containing 25 µg of Hg(II) was filled with varying amounts of the appropriate metal ions to form ternary synthetic mixtures. After adding potassium iodide, the solution was diluted to the calibration mark, resulting in a final KI concentration of 0.10 mol $L^{-1}$. The contents were mixed thoroughly to ensure complete formation of the Hg(II)– HNMHC complex, which was subsequently analyzed using spectrophotometry at 368 nm. The obtained results are presented in Table 5.

**Table 5: Extraction, separation and determination of Hg (II) from ternary synthetic mixtures.**

Mercury (II): 25 µg $ml^{-1}$, KI: 0.10 mol $L^{-1}$, HNMHC: 0.004 mol $L^{-1}$, λmax: 368 nm

| Composition (µg) | RSD (%) | Recovery[a] (%) |
|---|---|---|
| Hg(II) 25; Cr(III)[*b] 80; Cd(II)[*a] 30 | 0.27 | 99.16 |
| Hg(II) 25; Co(II)50; Ni(II)100 | 0.35 | 99.44 |
| Hg(II) 25; Co(II)50; Zn(II)[*b] 100 | 0.27 | 97.48 |
| Hg(II) 25; Al(III)[*a]100; N (II)100 | 1.23 | 98.60 |
| Hg(II) 25; Pb(II)[*b] 25; Co(II)50 | 0.26 | 99.44 |

a: Average four determinations

### Extraction, separation and determination of mercury (II) in Environmental waters

Individually environmental water sample (250 mL) was filtered using Whatman No. 40 filter paper and then shifted into a 500 mL distillation flask. To this, 10 mL of concentrated nitric acid was added. Following the standard protocol, the material was digested in the presence of excess potassium permanganate. [11] After cooling, the solution was neutralized with dilute ammonium hydroxide. After digestion, the mixture was put into a 25 mL volumetric flask and made up with deionized water. The mercury concentration in the water samples was subsequently determined using the recommended analytical method. The obtained results are presented in Table 6.

**Table 6: Extraction, separation and determination of Hg (II) from Environmental sample.**

Mercury (II): 25 µg $ml^{-1}$, KI: 0.10 mol $L^{-1}$, HNMHC: 0.004 mol $L^{-1}$, λmax: 368 nm

| Samples | Mercury Added (µg) | Mercury found (µg) | RSD (%) | Recovery[a] (%) |
|---|---|---|---|---|
| **Pravara River** | 25 | 24.79 | 0.66 | 99.16 |
| **Lake Water** | 25 | 24.86 | 0.87 | 99.44 |
| **Ground Water** | 25 | 24.72 | 0.67 | 98.88 |

a: Average three determinations

## Conclusion:

The proposed extractive spectrophotometric method offers a rapid, simple, and highly selective strategy for the trace determination of mercury (II) using 2- [(3-hydroxy-4-nitrophenyl) methylidene] hydrazine-1-carbothioamide as a chromogenic reagent. The method is based on the formation of a stable Hg (II)–HNMHC complex in the presence of potassium iodide, which is effectively extracted into n-butanol and measured at 368 nm. It exhibits good linearity, high sensitivity, and a low detection limit over the investigated concentration range. The procedure shows excellent tolerance to common interfering ions, with successful masking ensuring enhanced selectivity. Its applicability was validated through analysis of synthetic mixtures and environmental water samples, yielding satisfactory accuracy and precision. Overall, the method is economical, efficient, and suitable for repetitive monitoring of mercury (II) at trace levels in environmental samples.

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**Data Availability:** The data that support the findings of this study are available on request.

## Declarations

**Conflict of Interest:** The authors declare no conflict of interest.

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